Stebbins, R. C. 1985. A Field Guide to Western Reptiles and Amphibians. Houghton Mifflin Company, Boston. 336 pp.

Steelman, G. M. 1939. *Oochoristica whitentoni*, a new anoplocephalid cestode from a land tortoise. Journal of Parasitology 25:479–482.

Yamaguti, S. 1961. Systema Helminthum. The Nematodes of Vertebrates. Vol. 3, Part 1. Interscience Publishers, New York. 679 pp.

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Research Note

Attraction of Amoebocytes to *Cyclocoelum oculeum* Rediae Entering the Snail Host

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ABSTRACT: Sixteen-millimeter cinephotomicrography was used to study the attraction of amoebocytes to *Cyclocoelum oculeum* rediae entering the snail host, *Gyraulus parvus*. As the redia pushed against the lining of the peripheral blood sinus, amoebocytes emerged from among epithelial cells lining the sinus 10–14 μm away and migrated to the area of penetration within 7–10 sec. When the redia broke through the sinus, the amoebocytes attached.

KEY WORDS: Trematoda, Cyclocoelidae, *Cyclocoelum oculeum, Gyraulus parvus*, redia, amoebocytes, 16-mm cinephotomicrography.

Cyclocoelum oculeum miracidia each contain a fully-formed redia (Palm, 1963). After attachment of the miracidium to the snail host, the activated redia leaves the miracidium within an hour and enters the snail. While studying this process using cinephotomicrography, it was noted that amoebocytes were attracted to the site of redial penetration.

Even though there is an extensive literature on amoebocytes and their relationship to helminths (Bayne, 1983; Cheng et al., 1969; Joky et al., 1985; Sullivan, 1988), there has never been any in vivo filming of the attraction and attachment of these cells to entering parasites.

Adult *C. oculeum* miracidia were collected from the nasal cavities and orbits of American coots (*Fulica americana*) and placed in sterilized aquarium water. Their uteri were dissected out and teased apart to release miracidia. Miracidia were then placed along with the recently dissected head-foot of *Gyraulus parvus* on a clean microscope slide in a drop of water and mounted with a coverslip. Filming was accomplished using a Bolex 16-mm movie camera with attached Nikon Cine Autotimer mounted on a Zeiss Universal Microscope. Photographs were taken at 1-sec intervals using Kodak Plus-X positive film. Selected frames were printed as negatives to retain detail. Line drawings from the same frames were produced to further clarify the relationships.

The most common attachment site of the miracidium was to the snail's tentacle. This organ is covered by ciliated epithelium underlain by dense connective tissue, peripheral blood sinuses, and a central artery. After attachment, the apical papilla of the miracidium elongated and pushed against the connective tissue surrounding the peripheral blood sinus. Following this the enclosed redia became very active. Eventually the apical papilla retracted into the miracidium and the redia broke through the miracidial membranes, moved past the retracted papilla, and pushed up against the peripheral blood sinus causing it to invaginate. Apparently aiding in the breakdown of the miracidial membranes and snail tissues were substances produced by the redial esophageal glands. These glands can be seen decreasing in volume as the redia escaped the miracidium and entered the snail. On numerous occasions while filming, and also when making observations, 1–3 amoebocytes could be seen emerging from among epithelial cells lining the blood sinus. One such example is depicted in Plate I, Figures 1–6 and Plate II, Figures 1–6

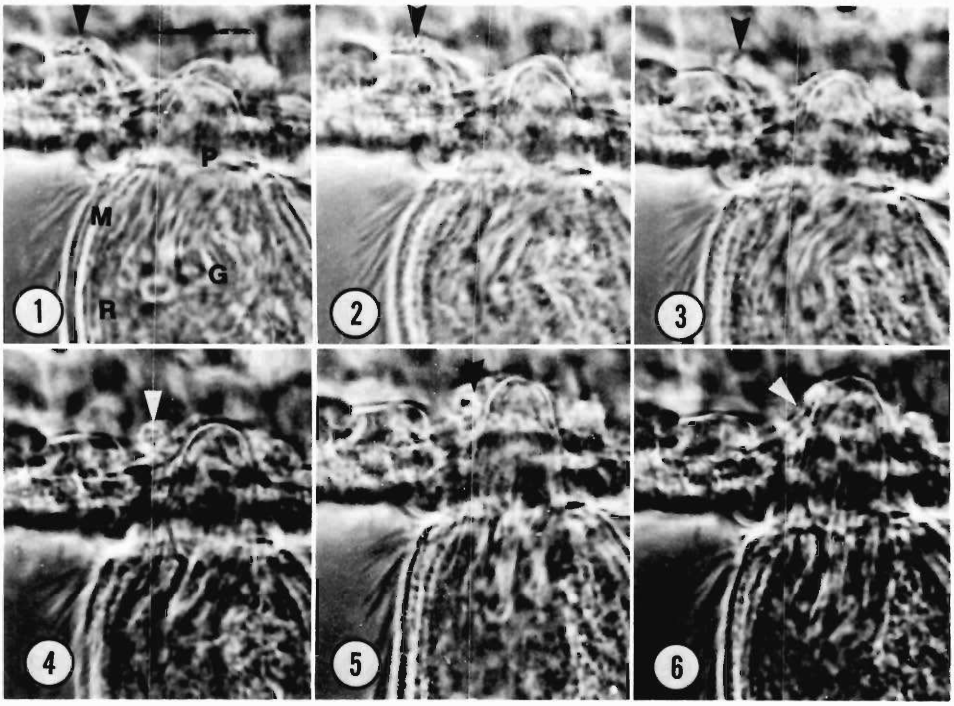

Plate I. Figures 1–6. ***Cyclocoelum oculeum*** **miracidium (m) containing a redia (r) with a pharynx (p) and esophageal glands (g). This sequence of Figures 1-6, reproduced from 16-mm film, shows the movement of, and attachment by an amoebocyte (arrow) to the entering redia. A decrease in size of the esophageal glands is noted. The gland contents may aid the redia during the penetration process. Scale bar = 14 μm.**

where amoebocytes at a distance of 10–14 μm from the redia migrated to the area of penetration within 7–10 sec. As soon as the redia broke through the blood sinus, amoebocytes attached. According to Cheng et al. (1969), there is no universal consensus in what tissues or organs the phagocytic cells originate. They may come from epithelial tissue of various organs, from mantle or lung connective tissue, from cells budding off the hepatopancreas, from fibroblasts in the mantle blood sinuses, and from the cellular reticulum found in the wall of the kidney near the pericardium. Some believe, according to Cheng et al. (1969), that connective tissue cells and those of the epithelium are capable of differentiating into amoebocytes. This study indicated that amoebocytes emerged from among epithelial cells lining the blood sinus. Whether they originated here is unclear. According to Bayne (1983), snail responses to trematode larval invasion may take 1 of 3 pathways: encapsulation with destruction; no cellular response; benign association of hemocytes with parasite surfaces. It appears that invasion of *G. parvus* by *C. oculeum* rediae follows the latter response.

It is difficult to assess the role the amoebocytes may play in protecting the snail from invasion by *C. oculeum* rediae because in the laboratory one can infect snails with large numbers (up to 30 or more) and the snails survive. No cellular reactions can be observed against this stage as determined by histochemistry (Taft, unpubl.). *Cyclocoelum oculeum* larvae are unusual among trematodes in that they are not very host specific, developing in snails of other genera including *Physa, Lymnaea,* and *Helisoma* (Taft, 1972). Whether or not a greater or lesser amoebocyte response occurs in snails of these genera to invading *C. oculeum* rediae might be tested in the laboratory.

The cyclocoelid–*Gyraulus* system is a useful model system in studying attraction and attachment of amoebocytes to rediae for the following reasons: 1. The snails are small and translucent;

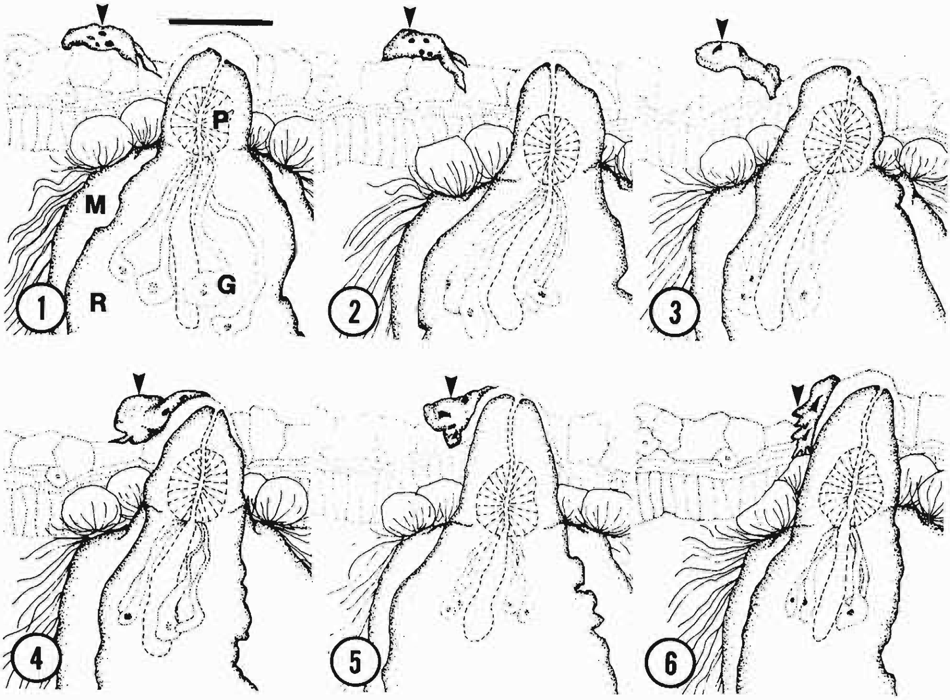

Plate II. Figures 1-6 are a series of line drawings of Plate I depicting the same events for greater clarity. Scale bar = 14 μm.

2. The rediae are large, thus making photography relatively easy; 3. Large numbers of miracidia can easily be obtained from 1 worm.

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Literature Cited

Bayne, C. J. 1983. Molluscan immunobiology. Pages 407–486 *in* A. S. M. Saleuddin and K. M. Wilbur, eds. Biology of Mollusca. Academic Press, New York.

Cheng, T. C., A. S. Thakur, and E. Rifkin. 1969. Phagocytosis as an internal defense mechanism in the Mollusca: with an experimental study of the role of leucocytes in the removal of ink particles in *Littorina scabra* Linn. Pages 546–563 *in* Proceedings of the Symposium on Mollusca, Part II. Marine Biology Association, India.

Joky, A., M. Matricon-Gondran, and J. Benex. 1985. Response of the amoebocyte-producing organ of sensitized *Biomphalaria glabrata* after exposure to *Echinostoma caproni* miracidia. Journal of Invertebrate Pathology 45:28–33.

Palm, V. 1963. Der Entwicklungzyklus von *Transcoelum oculeus* (Kossack, 1911) Witenberg, 1934 (family Cyclocoelidae) aus dem Blasshuhn (*Fulica atra* L.). Zeitschrift für Parasitenkunde 22:560–567.

Sullivan, J. T. 1988. Hematopoiesis in three species of gastropods following infection with *Echinostoma paraensei* (Trematoda: Echinostomatidae). Transactions of the American Microscopical Society 107:355–361.

Taft, S. J. 1972. Aspects of the life history of *Cyclocoelum oculeum* (Trematoda: Cyclocoelidae). Journal of Parasitology 58:882–884.